K 122420

510(k) SUMMARY

NOV 28 2012

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92

Submitter's name:	Diazyme Laboratories
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Date the Summary was Prepared:	October 17, 2012
Name of the Device	25-OH Vitamin D EIA Kit 25-OH Vitamin D EIA Control Kit
Trade Name:	25-OH Vitamin D EIA Kit 25-OH Vitamin D EIA Control Kit
Common/Usual Name	Vitamin D Assay
Device Classification Name	Vitamin D Test System
Product code:	MRG – Vitamin D Test System JJX – Single (specified) Analyte Controls (Assayed and Unassayed)
Panel:	Chemistry (75)
Submission Type	510k
Regulation Number	21 CFR 862.1825 – Vitamin D Test System

	21 CFR 862.1660 – Quality Control material (Assayed and Un-assayed)
Device Class	II (Assay) I (Control)
Predicate Device:	The 25-OH Vitamin D EIA Test Kit and Control Kit is substantially equivalent to the currently marketed 25-OH Vitamin D EIA (k021163).
Manufacturing Address	Diazyme Laboratories 12889 Gregg Court Poway, CA 92064 USA
Establishment Registration	2032900

DESCRIPTION OF THE DEVICE

The Diazyme 25-hydroxy (25-OH) Vitamin D EIA uses the enzyme immuno-assay technology (EIA). It is based on the competition (for a 25-OH Vitamin D antibody) between a 25-OH Vitamin D conjugate coated on a microplate and the 25-OH Vitamin D content of a serum sample. Vitamin D samples are first extracted for 15 min at room temperature using reagent EX and deep-well pre-dilution strips. The extracted Vitamin D samples are then transferred to the coated microplate wells and the addition of reagent R1 (containing an antibody for 25-OH Vitamin D) allows for the competition to proceed. After a first incubation, the microplate is washed and reagent R2 (HRP-labeled secondary antibody) is added. Following a second incubation and a washing step, reagent R3 (TMB substrate) is added. After a final incubation step, the reaction is stopped by adding the STOP solution and the colorimetric signal of the microplate is measured at 450 nm (primary wavelength) to which the background signal can be subtracted by using a secondary wavelengths (620 to 650 nm). The 25-OH Vitamin D concentration of a patient sample is inversely proportional to the measured absorbance at 450 nm. The whole assay procedure takes about two hours. 25-OH Vitamin D EIA calibrator set is intended for use as a calibration for the Diazyme 25-OH Vitamin D EIA kit only. Six calibration levels are needed for each run. Calibrators are treated exactly the same as patient samples.

25-OH Vitamin D EIA 2-point control kit is intended for use with the Diazyme 25-OH Vitamin D EIA kit only. Controls are treated exactly the same as patient samples. The quality controls assist laboratory users in verification steps ensuring that the assay reagents are functioning correctly. Users are instructed to verify the calibration curve with the controls.

INDICATIONS FOR USE

The Diazyme 25-OH Vitamin D EIA Kit is designed for the quantification of total 25-OH Vitamin D in human serum and plasma. The assay results are to be used in parallel with other clinical data to assess the Vitamin D status of a patient. For *in vitro* diagnostic use only.

The 25-OH Vitamin D EIA Control Kit is intended for use as quality controls for the Diazyme 25-OH Vitamin D EIA Kit only. For *in vitro* diagnostic use only.

Table 1 Summary of Assay Kit Components

IDS 25-OH Vitamin D EIA (predicate k021163)	**Diazyme 25-OH Vitamin D EIA**
Kit can be only used for the 25-OH Vitamin D quantification on microplate readers.	Kit can be only used for the 25-OH Vitamin D quantification on microplate readers
Antibody Coated Plate (MICROPLAT) • Microplate with 25-OH Vitamin D sheep polyclonal antibody linked to the inner surface of the polystyrene wells, 12 x 8 well strips in a foil pouch with desiccants.	Coated Microplate • Microplate with a 25-OH Vitamin D derivative linked to the inner surface of the polystyrene wells, 12 x 8 well strips in a foil pouch with desiccants.
Adhesive plate sealer • 8 per kit	N/A
Dissociation buffer (25-D Biotin) 1 bottle • Proprietary reagent for dissociating Vitamin D • 25-OH Vitamin D labeled with Biotin • Stabilizers	Extraction solution (EX) 1 bottle • Extraction solution containing organic solvents.
Wash Buffer (WASHBUF) 1 bottle • Phosphate buffered saline containing Tween	Wash Buffer (Wash 20X) 1 bottle • A 20X concentrate of a phosphate buffered saline containing Tween 20.
Enzyme Conjugate (ENZYMCONJ) 1 bottle • Phosphate buffered saline containing avidin linked to horseradish peroxidase, protein, enzyme stabilizers and preservatives.	Reagent 1 1 bottle • Phosphate buffered saline containing a sheep monoclonal antibody specific to 25-OH Vitamin D, stabilizers and preservatives.
N/A	Reagent 2 1 bottle • Phosphate buffered saline containing an anti-sheep IgG linked to horseradish peroxidase, stabilizers and preservatives.

TMB substrate (SUBS) 1 bottle • Proprietary aqueous formulation of tetra-methylbenzidine (TMB) and hydrogen peroxide.	Reagent 3 1 bottle • Proprietary aqueous formulation of tetra-methylbenzidine (TMB) and hydrogen peroxide.
STOP solution (HCl) 1 bottle • Concentrated solution of hydrochloric acid HCl.	STOP solution (HCl) 1 bottle • Concentrated solution of hydrochloric acid HCl.
Calibrator set	Calibrator set
1 x 1.0 mL Calibrator 0 1 x 1.0 mL Calibrator 1 1 x 1.0 mL Calibrator 2 1 x 1.0 mL Calibrator 3 1 x 1.0 mL Calibrator 4 1 x 1.0 mL Calibrator 5 1 x 1.0 mL Calibrator 6	1 x 1.0 mL Calibrator 1 1 x 1.0 mL Calibrator 2 1 x 1.0 mL Calibrator 3 1 x 1.0 mL Calibrator 4 1 x 1.0 mL Calibrator 5 1 x 1.0 mL Calibrator 6
Control set	Control set
1 x 1.0mL Control 1 1 x 1.0mL Control 2	1 x 1.0mL Control 1 1 x 1.0mL Control 2

PERFORMANCE TESTING SUMMARIES ON DYNEX MICROPLATE READER

Precision Study

The precision of the Diazyme 25-OH Vitamin D EIA was evaluated according to the Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline.

The precision of the Diazyme 25-OH Vitamin D Enzyme-Immuno-Assay (25-OH Vitamin D EIA) was evaluated according to the Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. A total of 10 precision levels were used in the study:

- Two serum controls (containing 26.0 ng/mL and 52.3 ng/mL).
- Eight serum samples distributed across the dynamic range of the assay.

Controls and samples were measured daily, over the span of 20 days. Each day, a set of four measurements was obtained for each specimen with two independent runs, each run being done in duplicates.

The mean value (Mean), standard deviation, within-run imprecision and total imprecision are calculated and summarized in the following tables:

Specimen	n	Mean (ng/mL)	Within-run SD (ng/mL)	Within-run CV (%)	Total SD (ng/mL)	Total CV (%)
Control #1	80	26.0	0.97	3.7	1.55	6.0
Control #2	80	52.3	1.43	2.7	3.93	7.5
Sample #1	80	23.3	1.65	7.1	1.89	8.1
Sample #2	80	40.0	3.70	9.2	3.75	9.4
Sample #3	80	52.8	1.18	2.2	4.03	7.6
Sample #4	80	69.3	1.94	2.8	6.03	8.7
Sample #5	80	84.1	4.69	5.6	6.33	7.5
Sample #6	80	101.4	4.65	4.6	5.68	5.6
Sample #7	80	116.8	4.89	4.2	5.77	4.9
Sample #8	80	116.2	2.98	2.6	6.43	5.5
Low Sample #1	80	10.5	1.39	13.3	1.84	17.6
Low Sample #2	80	12.7	1.14	9.0	2.04	16.0

Linearity/Reportable Range

To establish the linearity of the assay, study design was used based on the CLSI protocol EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: a Statistical Approach: Approved Guideline.*

Eleven levels of linearity were prepared by diluting a high serum sample containing 145.6 ng/mL of 25-OH Vitamin D with a low serum sample containing 5.0 ng/mL of 25-OH Vitamin D, as measured by a the predicate device. The linearity levels were tested with the Diazyme 25-OH Vitamin D EIA, in triplicates. The results were processed using the EP Evaluator Software (Version 8.0) parameterized to an allowable systematic error of 7.8%. The assay was found to be linear between 6.7 ng/mL and 143.6 ng/mL. However, because the limit of quantitation of the assay was found to be 8.3 ng/mL, the claimed linearity range for the Diazyme 25-OH Vitamin D EIA is therefore 8.3 ng/mL to 143.6 ng/mL.

LoB/LoD/LoQ

The Limit of Blank (LoB), the Limit of Detection (LoD) and the Limit of Quantitation (LoQ) of the Diazyme 25-OH Vitamin D assay on microplate were determined according to CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation. The following are the limits determined with the Diazyme 25-OH Vitamin D EIA:

LoB = 3.0 ng/mL

LoD = 5.6 ng/mL

LoQ = 8.3 ng/mL

Analytical specificity

Interference Study

The Diazyme 25-OH Vitamin D EIA was subjected to an interference study according the CLSI EP7-A2 protocol. The following substances normally present in the blood produced less than 10% deviation when tested at levels equal to the concentrations listed below:

Interference	Concentration
Ascorbic Acid	10 mM
Free Bilirubin	40 mg/dL
Conjugated Bilirubin	40 mg/dL
Triglyceride	450 mg/dL
Hemoglobin	500 mg/dL

Cross Reactivity

Specificity of the Diazyme 25-OH Vitamin D EIA Assay was determined by adding Vitamin D metabolites to serum pool samples. Based on the results summarized in table below, the assay did not cross react with Vitamin d2 and Vitamin D3 and the assay recovers both 25-OH Vitamin D2 and 25-OH Vitamin D3 similarly. Although, the vitamin D metabolites 1, 25-OH and 24R, 25-OH showed cross reactivity as indicated below, these metabolites do not interfere with assay results when tested up to 100X the normal levels found in human sera.

Cross-reactant	Unspiked Vitamin D (ng/mL)	Spiked Vitamin D (ng/mL)	% Cross-reaction*
25-OH Vitamin D3 (tested at 63 ng/ml)	24.4	87.4	100.0%
25-OH Vitamin D2 (tested at 63 ng/ml)	24.4	85.4	96.8%
Vitamin D3 (tested at 63 ng/ml)	24.4	24.2	-0.3%
Vitamin D2 (tested at 63 ng/ml)	24.4	22.3	-3.3%
1;25-OH Vitamin D3 (tested at 63 ng/ml)	24.4	84.1	94.8%
1,25-OH Vitamin D2 (tested at 63 ng/ml)	24.4	62.0	59.7%
24R,25-OH Vitamin D3 (tested at 56 ng/mL)	9.8	23.2	23.9%
3-epi-25-OH Vitamin D3 (tested at 57 ng/ml)	21.2	69.6	84.9%
3-epi-25-OH Vitamin D2 (tested at 57 ng/ml)	21.2	59.9	67.9%

*Cross-reactivity was calculated as (spiked sample value - unspiked sample value/concentration spiked) *100

Comparison Studies

Method Comparison

Human serum samples were tested with the Diazyme 25-OH Vitamin D EIA and the obtained results were compared to the predicate method. A total of 58 serum samples were used in this experiment. To ensure that the tested concentrations of 25-OH Vitamin D were distributed across the reportable dynamic range, 7 samples in the set were spiked with 25-OH Vitamin D stock solution of 25-OH Vitamin D3 and measured with the Diazyme and the IDS 25-OH Vitamin D assays. Using this study, we found that the Diazyme 25-OH Vitamin D EIA correlated with the predicate method with the following results:

	Serum Samples
n	58
Slope	0.941
Intercept	+1.448
Correlation coeffi-	0.930
Range of values	11.9 ng/mL- 131.5 ng/mL

Matrix Comparison

To evaluate the effect of anticoagulants, the Diazyme 25-OH Vitamin D EIA was used to measure the 25-OH Vitamin D concentrations of matched sets of serum, K3-EDTA plasma and Li-Heparin plasma. The reported values for each sample and for each matrix were obtained from single measurements. The total number of matched sets tested was 66. In order to cover the claimed measuring range for each matrix, seven spiked patient samples were included in the study.
Linear regression of the "Li-Heparin plasma versus Serum" data yielded the following results: y = 0.993 x + 1.855 and R2 = 0.961.
Linear regression of the "K3-EDTA plasma versus Serum" data yielded the following results: y = 1.006x + 2.901 and R2 = 0.967.

Reference Range Study

To determine a reference range for the Diazyme 25-OH Vitamin D EIA, the 25-OH Vitamin D serum concentrations of a US population of 157 apparently healthy individuals were measured with the Diazyme method. The individual patient serum samples used in this study were obtained from certified commercial sources (ProMedDx, LLC and Dx Biosamples). Forty seven (47) samples from Pennsylvania (Northern U.S.) were collected from an FDA Licensed Donor Center with informed consent. Fifty six (56) samples from Tennessee (Central U.S.) and Fifty four (54) samples from Texas (Southern U.S.) were collected according to an IRB approved protocol.

All participating individuals met the following inclusion conditions:

- The age of all individuals was within the 21-80 years old range.
- Individuals were from three different geographical locations: 47 from Pennsylvania (Northern US), 56 from Tennessee (Central US) and 54 from Texas (Southern US).
- All samples were collected during the months of October and November (fall season).
- The studied population consisted of 72 light skin individuals (46%) and 85 dark skin individuals (54%).
- 155 individuals (98.7%) did not take any artificial Vitamin D supplements. Two individuals (1.3%) did take some Vitamin D supplements but did not exceed the daily dose of 2000 IU.
- All 157 individuals did not have any family history of parathyroid or calcium regulatory disease.
- All 157 individuals did not have any history of kidney disease, GI disease, liver disease, calcium-levels related disease, thyroid disease, parathyroid disease, calcium related disease, seizures, chronic disease or bariatric surgery.
- All 157 individuals were not currently taking any medications that are known to affect absorption or catabolism of Vitamin D (including cholesterol absorption inhibitors such as Vytorin®, Inegy™ or Zetia; anticonvulsants such as Neurontin, Depakine® and Trileptal; glucocorticoids such as Cortisol, Prednisone and Dexamethasone; HAART (AIDS treatment) or antirejection medications.

Analysis of the reference range study data yielded the following results:

- Lowest 25-OH Vitamin D concentration: 8.4 ng/mL.
- Highest 25-OH Vitamin D concentration: 61.3 ng/mL.
- Median 25-OH Vitamin D concentration: 29.1 ng/mL
- Observed range (2.5th to 97.5th percentile): 12.0 to 55.0 ng/mL.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-002

November 28, 2012

Diazyme Laboratories
c/o Dr. Abhijit Datta
12889 Gregg Court
Poway, CA 92064

Re: k122420
Trade/Device Name: Diazyme 25-OH Vitamin D EIA Kit
Diazyme 25-OH Vitamin D EIA Control Kit
Regulation Number: 21 CFR 862.1825
Regulation Name: Vitamin D Test System
Regulatory Class: Class II
Product Code: MRG, JJX
Dated: October 16, 2012
Received: October 18, 2012

Dear Dr. Datta:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set

forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostics and Radiological Health at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Carol C. Benson for

Courtney H. Lias, Ph.D
Director
Division of Chemistry and Toxicology Devices
Office of *In Vitro* Diagnostics and
Radiological Health
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): k122420

Device Name: Diazyme 25-OH Vitamin D EIA Kit and Diazyme 25-OH Vitamin D EIA Control Kit

Indications for Use:
The Diazyme 25-OH Vitamin D EIA Kit is designed for the quantification of total 25-OH Vitamin D in human serum and plasma. The assay results are to be used in parallel with other clinical data to assess the Vitamin D status of a patient. For *in vitro* diagnostic use only.

The 25-OH Vitamin D EIA Control Kit is intended for use as quality controls for the Diazyme 25-OH Vitamin D EIA Kit only. For *in vitro* diagnostic use only.

Prescription Use ___X___ (21 CFR Part 801 Subpart D) And/Or Over the Counter Use ____. (21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostics and Radiological Health (OIR)

Yung Chan

Division Sign-Off
Office of In Vitro Diagnostics and Radiological Health

510(k) k122420